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KINETICS OF DISTRIBUTION OF RADIOACTIVE PERCHLORATE IN RAT AND GUINEA-PIG THYROID GLANDS

SIEN YAO CHOW* AND D. M. WOODBURY†

Department of Pharmacology, University of Utah College of Medicine, Salt Lake City, Utah 84112, U.S.A.

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SUMMARY

The thyroidal uptake of $^{36}ClO_4^-$ and the effect of stable perchlorate ion and functional state of the thyroid, such as after long-term administration of propylthiouracil (PTU) or after hypophysectomy, on the uptake of $^{36}ClO_4^-$ were studied in rats and guinea-pigs. Based on the analysis of uptake curves, radioactive inulin and sulphate spaces, and histological measurements of compartmental sizes of the thyroid, the relative concentrations of $^{36}ClO_4^-$ in the various compartments were calculated and compared with those of $^{36}Cl^-$. The passage of $^{36}ClO_4^-$ and $^{36}Cl^-$ across both the basal and the apical membranes of the follicular cells in the different conditions were analysed. The results suggest that $^{36}ClO_4^-$ is concentrated in the lumen of the thyroid by a two-step process. It is actively transported across the basal cell membrane from the interstitial fluid into follicular cells, and is further concentrated in passage across the apical membrane into the lumen. The evidence suggests that the latter process is also an active one.

INTRODUCTION

Perchlorate is a monovalent anion that completely inhibits active uptake of iodide by the thyroid gland. It is also concentrated by the thyroid (Wyngaarden, Wright & Ways, 1952; Anbar, Guttmann & Lewitus, 1959; Lewitus, Guttmann & Anbar, 1962; Chow, Chang & Yen, 1969). Thus iodide and perchlorate are probably transported by the same carrier system. However, the kinetics of its uptake and the effect of the stable anion and that of the functional state of the thyroid, such as that after long-term administration of propylthiouracil (PTU) or after hypophysectomy, on the uptake of perchlorate by the thyroid gland have not been investigated.

One of the unsolved problems of active anion transport in the thyroid cells is the location of the carrier system in thyroid follicular membranes. Although some evidence (Andros & Wollman, 1964, 1967; Tong, 1964) suggests that the outer or basal thyroid cell membrane is the site of the active transport of iodide, it is not clear

* Present address: Department of Biophysics, National Defense Medical Center, Taipei, Taiwan, China.

† Recipient of a Research Career Award (5-K6-NB-13-838), National Institutes of Health, U.S.P.H.S.

whether the inner or apical membrane is also involved. Since perchlorate, unlike iodide, is not organically bound by the thyroid gland and does not undergo metabolic changes in the gland (Anbar *et al.* 1959; S. Y. Chow & L. R. Chang, unpublished observations), it provides a useful substance for studying the mechanism of active anion transport by the thyroid gland. Based on a kinetic study of perchlorate uptake by the thyroid gland and on a comparison of perchlorate distribution with that of chloride in the stromal, luminal and cellular compartments of the thyroid under different conditions, the passage of anions across both the basal and the apical membranes has been analysed. In addition, methods for calculating the concentration of anions in various compartments of the thyroid glands are presented.

MATERIALS AND METHODS

Male Sprague–Dawley rats and Hartley guinea-pigs were used. They were maintained on Purina laboratory chow and water *ad libitum*. Three experiments were performed as follows:

Experiment 1. Two hundred and ten rats (226 ± 4 g.) were used. All animals were functionally nephrectomized by ligating the renal pedicle of both kidneys 24 hr. before they were killed. They were divided into three groups. In each group, 50 rats received radioactive perchlorate. A $K^{36}ClO_4$ solution (0·5 μc/rat) containing a specified amount of stable $KClO_4$ was administered i.p. from 2 to 240 min. before killing the animals. Solutions of $K^{36}ClO_4$ containing different amounts of stable perchlorate were prepared by diluting the stock radioactive solution and adding adequate amounts of stable salt to make the final solution for injection. Twelve rats received either [^{14}C]inulin (5μc), $^{35}SO_4^{-2}$ (20 μc) or $^{36}Cl^-$ (0·5 μc) i.p. 2 hr. before being killed. The other animals were used for histological measurements and for obtaining the self-absorption correction of radioisotopes in the thyroid and plasma; they received no radioactive compound. Each animal was killed with ether anaesthesia according to a pre-set schedule. Samples of thyroid gland and blood were taken for radioactivity studies, and for determinations of water and electrolyte content. Blood was withdrawn from the end of the descending aorta into an heparinized syringe.

Experiment 2. Ninety-four intact (304 ± 4 g.) and ten hypophysectomized (169 ± 3 g.) rats were used. Half the intact animals were treated with PTU for 2 weeks. PTU was dissolved in the drinking water (0·1%), and dextrose was added to make the water sweet. Hypophysectomy was performed 4 weeks before the experiment. All animals were functionally nephrectomized as described above 24 hr. before being killed. $K^{36}ClO_4$ (0·5 μc/rat) was administered i.p. to 72 intact (36 control and 36 PTU-treated) and ten hypophysectomized rats from 3 min. to 24 hr. before killing. The other animals (half control and half PTU-treated) received either [^{14}C]inulin, $^{35}SO_4^{-2}$, or $^{36}Cl^-$ or no radioactive compounds, as described for expt 1. The animals were killed with ether anaesthesia according to the schedule. Thyroid gland and blood samples were taken.

Experiment 3. Eighty-two guinea-pigs (394 ± 11 g.) were used. Half were treated with PTU (0·1% in drinking water) for 2 weeks. All animals were functionally nephrectomized as was done in rats 24 hr. before being killed. The experimental design with respect to administration of radioisotopes and the time schedule was

same as that for expt 2, except the animals were killed in sodium pentobarbitone anaesthesia.

All thyroid and blood samples were prepared, counted, and analysed by procedures described previously (Chow & Woodbury, 1965).

In each experiment, 2–4 animals were prepared in exactly the same manner as were the groups for radioactivity studies. Thyroid glands were isolated, fixed in Bouin's solution, embedded in paraffin, sectioned at 5–6 μ and stained with haematoxylin and eosin. Thickness of follicular cells and diameter of follicles of the stained thyroid sections were measured by use of a light microscope ($\times$ 440) with the aid of an eye piece micrometer. Forty follicles from different areas of 6–8 sections were measured in each animal. The means of the cell and follicular measurements from each group were taken, and percentages of the volume of the follicular lumen and cells were then calculated on the assumption that follicles are spheres. No correction was made for mid-portion section (Chow & Woodbury, 1965). Subsequent calculations are described in the Appendix.

The source and specifications of the radioactive compounds used were as follows: [^{36}Cl]potassium perchlorate (sp. act.: 533 μc/m-mole) and [^{36}Cl]NaCl (sp. act. : 690 μc/m-mole) were obtained from the Radiochemical Centre, Amersham, England, [^{14}C]carboxyl inulin (sp. act.: 100 μc/28·3 mg.) from New England Nuclear Corp., U.S.A. and [^{35}S]H_2SO_4 in HCl (carrier free) from Tracerlab, U.S.A.

RESULTS

Uptake of radioactive perchlorate by the thyroid glands of rats injected with stable perchlorate

Figure 1A shows the thyroid uptake curves of $^{36}ClO_4^-$ in rats that received stable perchlorate in doses of 0·005, 0·1, or 2·0 m-mole $KClO_4$/kg. The ratios of $^{36}ClO_4^-$ in thyroid gland and interstitial fluids were calculated according to the following formula and were plotted semilogarithmically as a function of time from 2 to 240 min. after injection of $^{36}ClO_4^-$.

$$\frac{\text{Thyroid gland}}{\text{Interstitial fluid}} = \frac{\text{Thyroid activity (counts/min./g.)} \times \text{plasma } H_2O \text{ (g./ml.)} \times 0{\cdot}95}{\text{Plasma activity (counts/min./ml.)}}$$

In Fig. 1A, the shape of thyroid $^{36}ClO_4^-$ uptake curves in rats that received 0·005, 0·1, or 2·0 m-mole $KClO_4$/kg. is similar. A plateau was reached in all curves at about 4 hr. after the injection of $^{36}ClO_4^-$. However, the maximal uptake of $^{36}ClO_4^-$ by the rat thyroid was inversely proportional to the amount of perchlorate received by the animal: the lower the dose of stable perchlorate, the higher was the $^{36}ClO_4^-$ uptake.

All three curves could be resolved graphically into two components (Fig. 1B, C and D). The half-time and zero-time intercept for each component of the individual uptake curves are shown on the corresponding curves. The thyroid:interstitial fluid ratios of [^{14}C]inulin, $^{35}SO_4^{-2}$ and $^{36}Cl^-$ for each group of animals are also presented in the same figure.

Changes of $^{36}ClO_4^-$ concentration in plasma and thyroid gland with time in the three groups of rats that received the different doses of stable perchlorate are shown

in Fig. 2. Both the plasma and thyroid levels of $^{36}ClO_4^-$ reached maximum at about 8–15 min. after injection of $^{36}ClO_4^-$ and this was maintained for 240 min. The plasma concentrations of $^{36}ClO_4^-$ in all three groups at the same time-period were not significantly different, while the thyroid concentration of $^{36}ClO_4^-$ varied markedly.

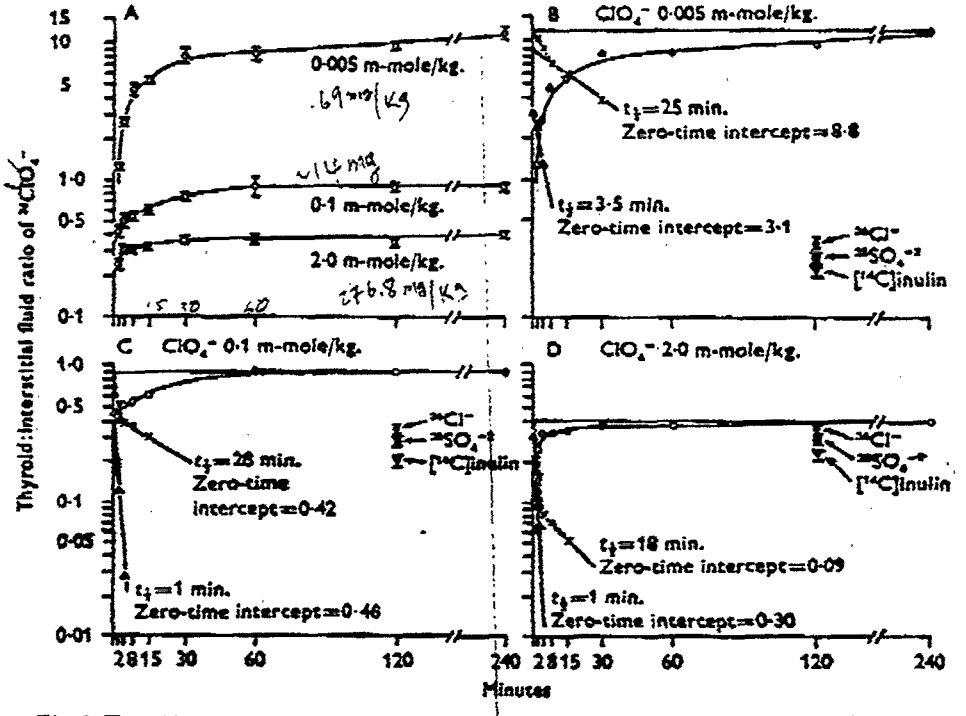

Fig. 1. Thyroid uptake curves of $^{36}ClO_4^-$ of rats that received 0·005, 0·1 or 2·0 m-mole $KClO_4$/kg. In A, the three uptake curves are drawn on the same graph. Each $^{36}ClO_4^-$ uptake curve in A is graphically resolved as indicated in B, C and D (see text for explanation). [^{14}C]Inulin, $^{35}SO_4^{-2}$, and $^{36}Cl^-$ spaces of the thyroid gland in each experiment are also shown in the corresponding graphs. In all graphs in this figure, ordinates are thyroid:interstitial fluid ratios of $^{36}ClO_4^-$; abscissa, time in minutes. In this and subsequent figures, each point represents the mean of 4–6 animals; the vertical bracketed lines indicate ± S.E.

Uptake of radioactive perchlorate by rat and guinea-pig thyroid glands in different functional states

The uptake of $^{36}ClO_4^-$ by the thyroid gland of control, PTU-treated, and hypophysectomized rats and of control and PTU-treated guinea-pigs is shown in Fig. 3. The thyroid:interstitial fluid $^{36}ClO_4^-$ ratios in each group of animals are plotted semilogarithmically as a function of time from 3 min. to 24 hr. after injection of $^{36}ClO_4^-$. [^{14}C]Inulin, $^{35}SO_4^{-2}$ and $^{36}Cl^-$ spaces of control and PTU-treated rat and guinea-pig thyroids are also shown in the same figure.

Figure 3 shows that the uptake of $^{36}ClO_4^-$ by the guinea-pig thyroid was greater than that of the rat thyroid. In both the guinea-pig and rat, PTU-treated animals had higher $^{36}ClO_4^-$ uptake values than the appropriate controls. In addition, the

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thyroid : interstitial fluid ratio of $^{36}ClO_4^-$ in hypophysectomized rats was much smaller than that of the other groups of animals and was the same as the thyroid $^{36}Cl^-$ space.

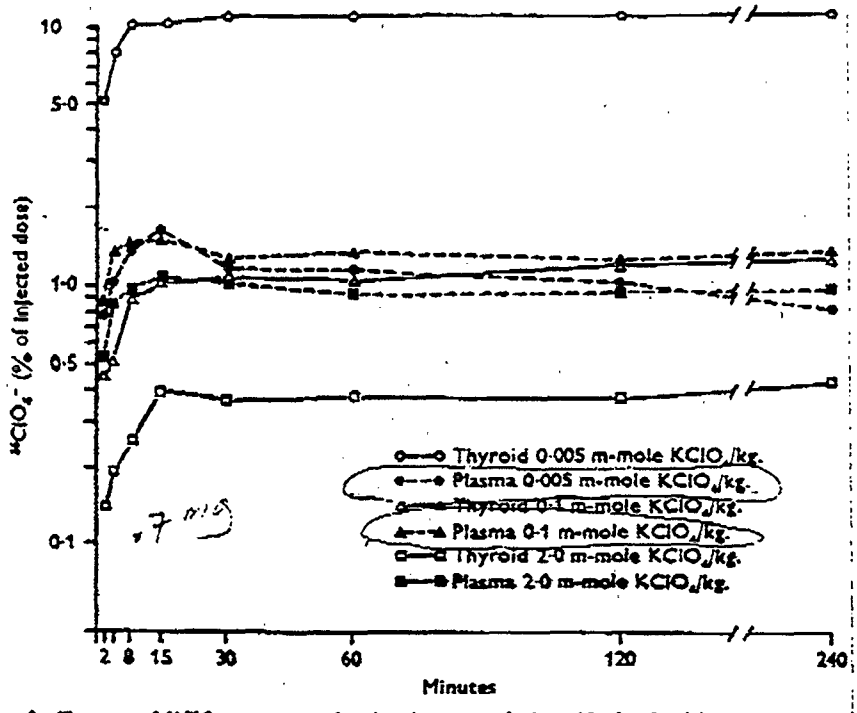

Fig. 2. Changes of $^{36}ClO_4^-$ concentration in plasma and thyroid gland with time in rats that received 0·005, 0·1 or 2·0 m-mole $KClO_4$/kg. Ordinate: percentage of injected dose of $^{36}ClO_4^-$ per ml. plasma or per g. of thyroid tissue. Abscissa: time in minutes.

In control and PTU-treated rats, the plateau in both curves was reached at about 4 hr. after the injection of $^{36}ClO_4^-$, while in guinea-pigs this took about 8 hr. All $^{36}ClO_4^-$ uptake curves in Fig. 3, except that of hypophysectomized rats, could be resolved graphically into two components. Because of limited space they are not shown in the figure. However, the half-time and the zero-time intercept for each component of the individual uptake curves are listed in Table 1. In hypophysectomized rats, the thyroid uptake curve of $^{36}ClO_4^-$ could not be resolved graphically because insufficient data were obtained at the early time-periods.

DISCUSSION

The uptake curves presented in Figs. 1 and 3 show that $^{36}ClO_4^-$ was concentrated by the thyroid gland of normal rats and PTU-treated rats that received smaller doses of stable perchlorate but not by the thyroid gland of normal rats that received 2 m-mole $KClO_4$/kg. and hypophysectomized rats that received 0·05 m-mole $KClO_4$/kg. The thyroid : interstitial fluid $^{36}ClO_4^-$ ratio in all groups of animals was considerably larger than the same ratio for $^{35}SO_4^{-2}$ and [^{14}C]inulin, substances that,

respectively, measure the stromal plus luminal volume and stromal volume of the thyroid gland (Chow, Jee, Taylor & Woodbury, 1965; Chow & Woodbury, 1965). Except in normal rats that received 2 m-mole $KClO_4$/kg. and in hypophysectomized rats that received 0·05 m-mole $KClO_4$/kg., the $^{36}ClO_4^-$ ratio was also larger than the $^{36}Cl^-$ ratio. Unlike the actively transported monovalent perchlorate anion,

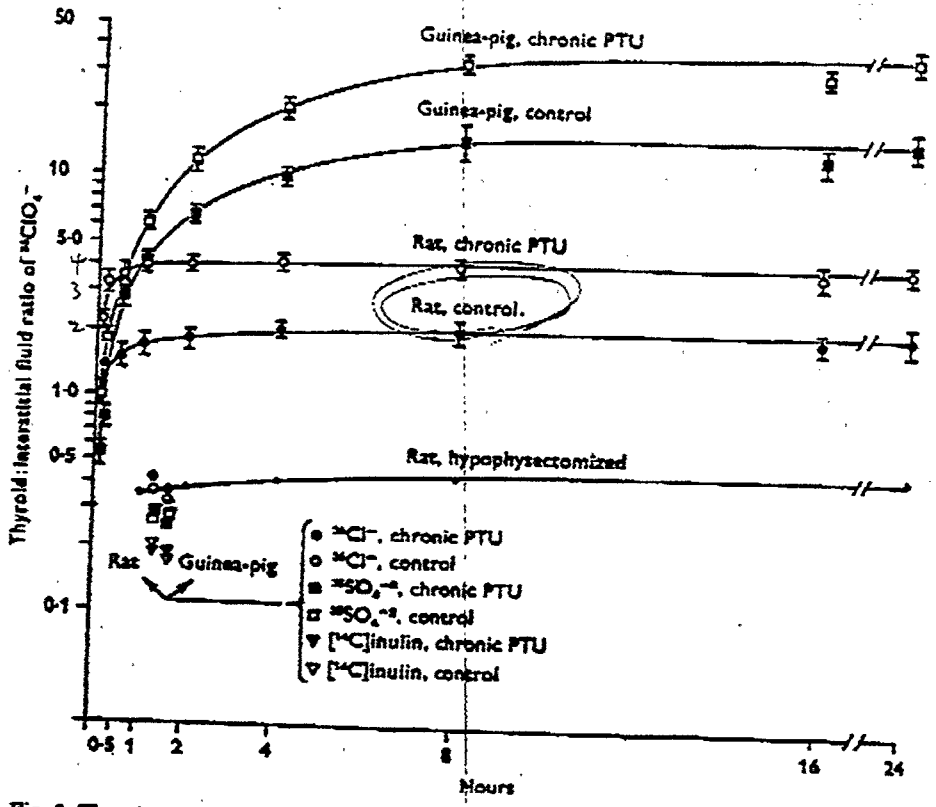

Fig. 3. Thyroid uptake curves of $^{36}ClO_4^-$ in control, propylthiouracil (PTU)-treated and hypophysectomized rats, and of control and PTU-treated guinea-pigs. [^{14}C]Inulin, $^{35}SO_4^{--}$, and $^{36}Cl^-$ spaces of the rat and guinea-pig thyroid gland, except those of hypophysectomized rats, are also shown in the same figure.

Table 1. *The half-time and the zero-time intercept of the two components of the $^{36}ClO_4^-$ uptake curves of rat and guinea-pig thyroid glands*

	Rat		Guinea-pig	
	Control	Chronic PTU	Control	Chronic PTU
Fast component:				
Zero-time intercept	1·2	3·3	1·2	2·0
$t_{\frac{1}{2}}$(min)	2	2	8	5
Slow component:				
Zero-time intercept	0·9	0·9	14·8	38
$t_{\frac{1}{2}}$(min)	32	50	160	240

See Materials and Methods for details of propylthiouracil (PTU) treatment.

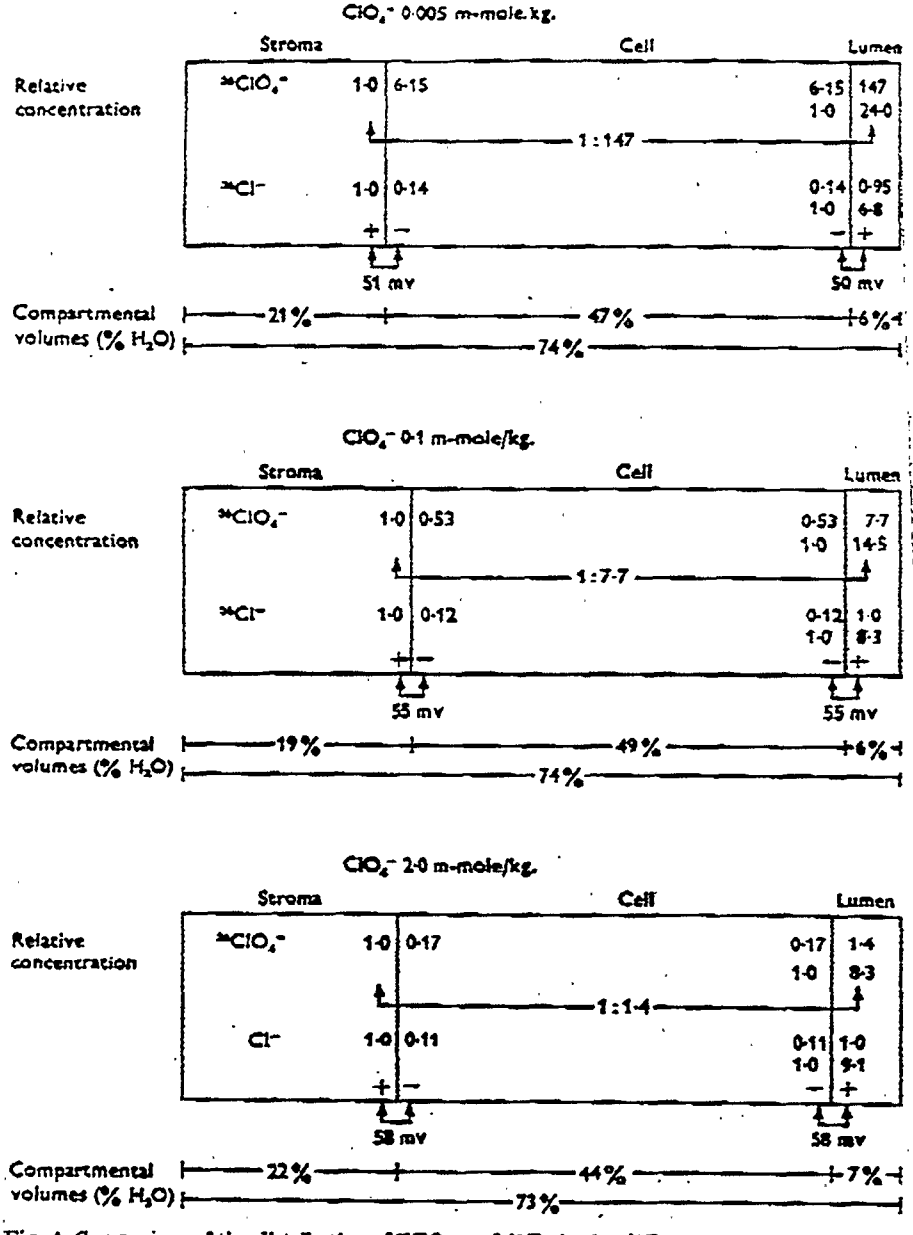

Fig. 4. Comparison of the distribution of $^{36}ClO_4^-$ and $^{36}Cl^-$ in the different anatomical compartments of the thyroid gland of rats that received 0·005, 0·1, or 2·0 m-mole $KClO_4$/kg. See text and Appendix for explanation and calculations.

chloride distributes passively across thyroid follicular cells in equilibrium with the transmembrane potential (Woodbury & Woodbury, 1963; Chow & Woodbury, 1965). The thyroid:interstitial fluid $^{36}ClO_4^-$ ratio of rats that received 2 m-mole/kg. stable perchlorate was almost equal to the $^{36}Cl^-$ space. This observation, combined with the results in hypophysectomized rats and with radioautographic evidence that $^{36}ClO_4^-$ is distributed like $^{36}Cl^-$ in the presence of 5 m-mole/kg. of stable perchlorate (S. Y. Chow & D. M. Woodbury, unpublished results), suggests that, when the active transport process is blocked, perchlorate is distributed passively like chloride across thyroid follicular membranes. This is also true for the monovalent iodide anion which distributes passively like chloride when its active transport is blocked (S. Y. Chow & D. M. Woodbury, unpublished observations).

Table 2. *Relative concentrations of $^{36}ClO_4^-$ and $^{36}Cl^-$ in various compartments of rat and guinea-pig glands*

Experimental animal*	Dose of $KClO_4$ (m-mole/kg.)	Ratios of relative concentrations: Cell:Stroma $^{36}ClO_4^-$	Cell:Stroma $^{36}Cl^-$	Lumen:Stroma $^{36}ClO_4^-$	Lumen:Stroma $^{36}Cl^-$	Lumen:Cell $^{36}ClO_4^-$	Lumen:Cell $^{36}Cl^-$
Rat	0·005	6·15	0·14	147	0·95	24·0	6·8
	0·01	2·2	0·13	48	1·0	21·6	7·7
	0·1	0·33	0·12	7·7	1·0	14·5	8·3
	2·0	0·17	0·11	1·4	1·0	8·3	9·1
	5·0	0·12	0·12	1·0	1·0	8·3	8·3
Rat	0·07	2·1	0·18	11	1·0	5·3	5·4
Rat, PTU	0·07	6·0	0·35	15	0·78	2·5	2·2
Guinea-pig	0·05	4·6	0·10	41	0·38	9·0	3·8
Guinea-pig, PTU	0·05	5·8	0·13	140	0·55	24·0	4·2

* See Materials and Methods for details of treatment with propylthiouracil.

The overall thyroid:interstitial fluid ratio for $^{36}ClO_4^-$ does not provide much information about the location of the sites of active transport or the relative concentrations of $^{36}ClO_4^-$ in the three anatomical compartments of the thyroid gland. However, a compartmental analysis of the uptake curves in different conditions, as in Fig. 1 and Table 1, together with the measurements of the [^{14}C]inulin and $^{35}SO_4^{-2}$ space and the histological measurements of compartmental sizes of the thyroid gland allows some deductions as to the site(s) of the active anion transport and the concentrations of this anion in the anatomical compartments. In all cases, the uptake curve of $^{36}ClO_4^-$ by the thyroid gland could be resolved into two components. Since the penetration of anions into the stromal compartment of the thyroid is rapid and this space occupies only about 20% of the thyroid weight ([^{14}C]inulin space), the first or fast component probably represents the rapid penetration of $^{36}ClO_4^-$ into both the interstitial and the cellular compartments. The cellular concentrations can then be calculated from the zero-time intercept of the fast component which represents the amount of $^{36}ClO_4^-$ in the combined stromal and cellular compartments. If the amount in the stromal compartment is subtracted (interstitial concentration × [^{14}C]inulin space) and the remainder divided by the fluid volume of thyroid follicular cells derived

from histological measurements, the concentration of $^{36}ClO_4^-$ in thyroid cellular fluid is obtained, as shown in Fig. 4.

The second or slow component of the uptake curves probably represents the rapid passage of $^{36}ClO_4^-$ across the apical membrane from the follicular cells to the lumen and also the slow equilibration of $^{36}ClO_4^-$ in the luminal fluid. This value divided by the histologically determined luminal fluid volume gives the concentration of $^{36}ClO_4^-$ in the thyroid luminal fluid. The relative $^{36}ClO_4^-$ concentrations in various thyroid compartments (interstitial fluid $^{36}ClO_4^-$ concentration taken as 1·0) derived from these calculations are shown in Fig. 4 for rats that received various doses of stable perchlorate. The distribution of $^{36}Cl^-$ in the three different thyroid compartments is also shown in Fig. 4 for the same groups of animals. The ratios of relative concentration of both $^{36}ClO_4^-$ and $^{36}Cl^-$ among the three thyroidal compartments in these experiments and some of our other observations are summarized in Table 2. The complete calculations for the relative concentrations of anions in the different compartments are shown in the Appendix.

In rats that received the smallest dose of stable perchlorate (0·005 m-mole/kg.), the concentration of $^{36}ClO_4^-$ in the lumen was 24 times higher than that in the cells and 147 times higher than in the interstitial fluid (see Table 2 and Fig. 4). These values are in contrast to the thyroid:interstitial fluid ratio for the whole gland of 11·9 (Fig. 1A). Thus, $^{36}ClO_4^-$ is concentrated in the lumen of the thyroid by a two-step process. Firstly, $^{36}ClO_4^-$ is actively transported across the basal cell membrane from the stromal fluid into the cellular fluid (cell:stroma ratio for $^{36}ClO_4^-$ was 6·15 as compared with 0·15 for $^{36}Cl^-$). This process moves the negatively charged $^{36}ClO_4^-$ against its electrical gradient of approximately 50 mv (Woodbury & Woodbury, 1963) since the cell is negatively charged as compared with the stroma. In the cellular fluid, $^{36}ClO_4^-$ then passes across the apical cell membrane into the lumen and it is further concentrated during this process. Since the inside of the follicular cells is negative to the lumen, the potential difference across the apical membrane, in contrast to that of the basal membrane, drives the $^{36}ClO_4^-$ into the positively charged luminal fluid. However, since the ratio between the cellular and luminal $^{36}ClO_4^-$ concentration was 24 as compared with 6·7 for $^{36}Cl^-$, it is likely that there is also an active process taking place across the apical cell membrane of the thyroid gland. The transmembrane potentials across each membrane, calculated from the results with $^{36}Cl^-$ on the basis that it is passively distributed across the thyroid cells, are approximately equal to the values determined by direct measurement with glass ultramicroelectrodes (Woodbury & Woodbury, 1963), and this result confirms the passive nature of Cl^- distribution across thyroid cells.

Since perchlorate is a potent inhibitor of the thyroidal transport system, it is not surprising that, when the dose of stable perchlorate is increased, the relative $^{36}ClO_4^-$ concentrations between the various thyroidal compartments are decreased and approach those of $^{36}Cl^-$ (Table 2). As would be expected, the membrane on the basal side of the follicular cell is more sensitive to the blocking effect of perchlorate than is the membrane on the apical side.

Treatment with PTU caused an overall increase in the uptake of $^{36}ClO_4^-$ by the thyroid gland of rats and guinea-pigs. The increased uptake is probably a result partly of the enhanced effect of the released thyroid-stimulating hormone (TSH) (due

to impaired synthesis of thyroid hormones) on the transport mechanism across thyroid cell membranes. This is indicated by the increase in the zero-time intercept values of the two components of the $^{36}ClO_4^-$ uptake curves in PTU-treated animals. However, there was a difference between the rat and the guinea-pig after PTU treatment. In rats, the increase in zero-time intercept values occurred only in the fast component, in guinea-pig the values of both components were increased. This variation may be due to the different changes in colloid content of the follicular lumen after PTU treatment in these species. Based on the $^{36}Cl^-$ results, the transmembrane potentials across thyroid follicular cells decreased (from 54 to 30 mv in rat and from 63 to 53 mv in guinea-pig experiment) after PTU treatment. The calculated decrease induced by PTU is in agreement with the observations by Woodbury & Woodbury (1963) that this drug and TSH markedly decrease the transmembrane potential as measured directly by glass ultramicroelectrodes.

The mechanism of the effect of TSH to increase ClO_4^- and I^- uptake by the thyroid gland is not clear at present, but a hypothesis that has some experimental support can be presented here. It has been shown that TSH increases the permeability of the thyroid cell membranes to Na^+ (Solomon, 1961) and to Cl^- and $SO_4^{=}$ (Chow & Woodbury, 1965), and lowers the transmembrane potential (Woodbury & Woodbury, 1963). The present results show that the overall uptakes of $^{36}ClO_4^-$ by the thyroid gland are increased by endogenous TSH as a result of chronic treatment with PTU of rats and guinea-pigs. If the half-times of the various components of the uptake curves are converted to K values ($K = 0{\cdot}693/t_{\frac{1}{2}}$), then the relative permeability constant (P) of the various components can be obtained by taking into account the volumes (V) in which the $^{36}ClO_4^-$ in each component are distributed ($P \propto KV$). The results show that PTU treatment increases the P value of the fast component, which represents passage of $^{36}ClO_4^-$ across the basal cell membrane of the thyroid gland, in both rats and guinea-pigs, but increases the P values of the slow component, which represents passage across the apical cell membrane, in guinea-pigs only. The data also show that the basal cell membrane has a higher permeability to $^{36}ClO_4^-$ than does the apical cell membrane. The increase in permeability to $^{36}ClO_4^-$ and the decrease in transmembrane potential, as calculated from $^{36}Cl^-$ distribution data after PTU treatment, would result in an additional amount of this anion entering the cells passively; this superimposed on the amount present as a result of active transport across the basal follicular membrane would increase markedly the concentration of $^{36}ClO_4^-$ in the cells. Evidence that most of the increase in cell $^{36}ClO_4^-$ after PTU treatment (or induced by TSH) is due to the increased permeability and depolarization is derived from a comparison of the distribution of $^{36}Cl^-$ and $^{36}ClO_4^-$. In PTU-treated animals, the increase in cellular $^{36}Cl^-$ over control values is 1·95-fold in rats and 1·3-fold in guinea-pigs; the comparable increase in cellular $^{36}ClO_4^-$ is 2·85-fold in rats and 1·25-fold in guinea-pigs. The increases for both ^{36}Cl and $^{36}ClO_4^-$ are very similar, especially in guinea-pigs. Thus, treatment with TSH or PTU does not have much effect on the active component of the $^{36}ClO_4^-$ transport process in the basal follicular membrane, and increases only that portion of the $^{36}ClO_4^-$ that is passively distributed, by increasing permeability and depolarizing the membrane.

The effect of TSH and PTU on transport at the apical follicular membrane is more complicated because of the different results obtained in rats and guinea-pigs.

As described before, the increased $^{36}ClO_4^-$ and $^{36}Cl^-$ concentration in cells would enhance the transport rate and thereby increase the concentration of these anions in the lumen. However, after PTU treatment, the ratios of lumen : cell for both $^{36}ClO_4^-$ and $^{36}Cl^-$ are decreased in rats and increased in guinea-pigs. This discrepancy in the two species is probably due to the difference in the colloid content in the follicular lumen and in their response to PTU. Rats have a much smaller luminal water volume than guinea-pigs. After PTU administration for 2 weeks, the volume of luminal water increased in the rat and decreased in the guinea-pig. This problem of the thyroid luminal water volume and electrolyte distribution is currently under investigation.

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APPENDIX

The values for the concentration of $^{36}ClO_4^-$ in each compartment of the thyroid gland (Fig. 4) are calculated from the zero-time intercept values of the two components of the uptake curves shown in Fig. 1 B, C, and D. The values for rats that received 0·005 m-mole $KClO_4$/kg. were calculated as follows:

Zero-time intercept of the fast component	3·1
Zero-time intercept of the slow component	8·8
Total	11·9
Total thyroid water	0·74
[^{14}C]inulin space (stromal volume)	0·21
Follicular water = total thyroid water − [^{14}C]inulin space	= 0·53
Histologically determined follicular cell volume	63%
Histologically determined luminal volume	37%

Assume the follicular cell water content is the same as that of other tissue cells in the body, i.e. 75 %. Follicular cell water volume = follicular cell water content × histologically determined follicular cell volume = 0·47. Luminal water volume = follicular water − cell water = 0·06. (Compared with the volume from the difference between $^{35}SO_4^{-2}$ and [^{14}C]inulin spaces, 0·264 − 0·210 = 0·054.) Assume stromal $^{36}ClO_4^-$ concentration $[^{36}ClO_4^-]_s$ = 1·0. Amount of $^{36}ClO_4^-$ in interstitial fluid = [^{14}C]inulin space × $[^{36}ClO_4^-]_s$ = 0·21.

If the fast component of $^{36}ClO_4^-$ uptake curve = stromal $^{36}ClO_4^-$ + follicular cell $^{36}ClO_4^-$, amount of $^{36}ClO_4^-$ in follicular cells = zero-time intercept value of the fast component − amount of $^{36}ClO_4^-$ in interstitial fluid = 3·10 − 0·21 = 2·89. Follicular cell $^{36}ClO_4^-$ concentration $[^{36}ClO_4^-]_c$ = amount of $^{36}ClO_4^-$ in follicular cells/cellular water volume = 2·89/0·47 = 6·15. If the slow component of $^{36}ClO_4^-$ uptake curve = luminal $^{36}ClO_4^-$, amount of $^{36}ClO_4^-$ in the lumen = zero-time intercept value of the slow component = 8·8. Luminal $^{36}ClO_4^-$ concentration $[^{36}ClO_4^-]_l$ = amount of $^{36}ClO_4^-$ in lumen/luminal water volume = 8·8/0·06 = 147.

The calculations for the $^{36}ClO_4^-$ distribution in thyroid gland of other groups of animals were done in the same way.

The $^{36}Cl^-$ distribution in different compartments of the thyroid gland of rats that received 0·005 m-mole $KClO_4$/kg. were calculated as follows: [^{14}C]inulin space, 0·210; $^{35}SO_4^{-2}$ space, 0·264; $^{36}Cl^-$ space, 0·334. Assume stromal $^{36}Cl^-$ concentration $[^{36}Cl^-]_s$ = 1·0. Amount of $^{36}Cl^-$ in interstitial fluid = $[^{36}Cl]_s$ × [^{14}C]inulin space = 0·21.

The ratio for the concentrations of $^{36}Cl^-$ in lumen to that in stroma (ρ) can be calculated by the following formula from the data of radioactive sulphate and inulin spaces and the histological measurements of the thyroid volumes (unpublished observations).

$$\rho = \frac{[^{36}Cl]_l}{[^{36}Cl^-]_s} = \sqrt{\left(\frac{^{35}SO_4^{-2}\text{ space} - [^{14}C]\text{inulin space}}{\text{Luminal water volume}}\right)}$$

$$= \sqrt{\left(\frac{0.264 - 0.21}{0.06}\right)} = 0.95.$$

Concentration of $^{36}Cl^-$ in lumen $[^{36}Cl^-]_l$ = 0·95 × 1·0 = 0·95.

Amount of $^{36}Cl^-$ in lumen = $[^{36}Cl^-]_l$ × luminal water volume = 0·95 × 0·06 = 0·057.

Amount of $^{36}Cl^-$ in follicular cells = total $^{36}Cl^-$ space − amounts of $^{36}Cl^-$ in interstitial fluid and in lumen = 0·334 − 0·21 − 0·057 = 0·067.

Concentrations of $^{36}Cl^-$ in follicular cells $[^{36}Cl^-]_c$ = amount of $^{36}Cl^-$ in cells/cellular water volume = 0·067/0·47 = 0·14.

The transmembrane potentials across the basal membrane (E_b) and across the apical membrane (E_a) based on the passive distribution of chloride and using the Nernst equation are as follows:

$$E_b = -60 \log \frac{[^{36}Cl^-]_s}{[^{36}Cl^-]_c} = -60 \log \frac{1}{0.14} = -51 \text{ mv.}$$

$$E_a = -60 \log \frac{[^{36}Cl^-]_l}{[^{36}Cl^-]_c} = -60 \log \frac{0.95}{0.14} = -50 \text{ mv.}$$